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<p><b>(21) International Application Number:</b> PCT/US00/07349</p> <p><b>(22) International Filing Date:</b> 17 March 2000 (17.03.00)</p> <p><b>(30) Priority Data:</b>          60/125,299                      19 March 1999 (19.03.99)                      US</p> <p><b>(71) Applicants (for all designated States except US):</b> SMITHK-LINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US). SMITHK-LINE BEECHAM PLC [GB/GB]; New Horizons Court, Great West Road, Brentford, Middlesex TW8 9EP (GB).</p> <p><b>(72) Inventors; and</b>  <b>(75) Inventors/Applicants (for US only):</b> HO, Yen, Sen [US/US]; 362 Old State Road, Berwyn, PA 19312 (US). HOLMES, Stephen, D. [GB/GB]; Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD (GB). TAYLOR, Alexander, H. [US/US]; 522 Westfield Drive, Exton, PA 19341 (US). ABDEL-MEGUID, Sherin, S. [US/US]; 236 Autumn Drive, Exton, PA 19341 (US).</p> <p><b>(74) Agents:</b> KEREKES, Zoltan et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).</p>		<p><b>(81) Designated States:</b> AE, AL, AU, BA, BB, BG, BR, CA, CN, CZ, EE, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MA, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, TZ, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b>  <i>With international search report.          Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p><b>(54) Title:</b> RECOMBINANT IL-18 ANTAGONISTS USEFUL IN TREATMENT OF IL-18 MEDIATED DISORDERS</p> <p><b>(57) Abstract</b></p> <p>Chimeric, humanized and other IL-18 mAbs, derived from high affinity neutralizing mAbs, pharmaceutical compositions containing same, methods of treatment and diagnostics are provided.</p>		

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RECOMBINANT IL-18 ANTAGONISTS USEFUL IN TREATMENT OF IL -18  
MEDIATED DISORDERS

5    FIELD OF THE INVENTION

The present invention relates generally to the field of antibodies and altered antibodies, useful in the treatment and diagnosis of conditions mediated by IL-18, and more specifically to mAbs, Fabs, chimeric and humanized antibodies.

10   BACKGROUND OF THE INVENTION

Human interleukin-18 is a recently identified cytokine that is synthesized as a biologically inactive 193 amino acid precursor protein (Ushio et al., J. Immunol. 156:4274, 1996). Cleavage of the precursor protein, for example by caspase-1 or caspase-4, liberates the 156 amino acid mature protein (Gu et al., Science 275:206, 15 1997; Ghayur et al., Nature 386:619, 1997), which exhibits biological activities that include the costimulation of T cell proliferation, the enhancement of NK cell cytotoxicity, the induction of IFN- $\gamma$  production by T cells and NK cells, and the potentiation of T helper type 1 (Th1) differentiation (Okamura et al., Nature 378:88, 1995; Ushio et al., J. Immunol. 156:4274, 1996; Micallef et al., Eur. J. Immunol. 20 26:1647, 1996; Kohno et al., J. Immunol. 158:1541, 1997; Zhang et al., Infect. Immunol. 65:3594, 1997; Robinson et al., Immunity 7:571, 1997). In addition, IL-18 is an efficacious inducer of human monocyte proinflammatory mediators, including IL-8, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) (Ushio, S. et al., J. Immunol. 156:4274-4279, 1996; Puren, A.J. et al., J. Clin. Invest. 25 10:711-721, 1997; Podolin et al., J. Immunol. submitted, 1999).

The previously cloned IL-1 receptor-related protein (IL-1Rrp) (Parnet et al., J. Biol. Chem. 271:3967, 1996) was recently identified as a subunit of the IL-18 receptor (Kd = 18 nM) (Torigoe et al., J. Biol. Chem. 272:25737, 1997). A second subunit of the IL-18 receptor exhibits homology to the IL-1 receptor accessory protein, and has been termed AcPL (for accessory protein-like). Expression of both 30 IL-1Rrp and AcPL are required for IL-18-induced NF- $\kappa$ B and JNK activation (Born et al., J. Biol. Chem. 273:29445, 1998). In addition to NF- $\kappa$ B and JNK, IL-18 signals through IL-1 receptor-associated kinase (IRAK), p56lck (LCK), and mitogen-activated protein kinase (MAPK) (Micallef et al., Eur. J. Immunol. 35 26:1647, 1996; Matsumoto et al., Biophys Biochem. Res. Comm. 234:454, 1997; Tsuji-Takayama et al., Biochem. Biophys. Res. Comm. 237:126, 1997).

Th1 cells, which produce proinflammatory cytokines such as IFN- $\gamma$ , IL-2 and TNF- $\beta$  (Mosmann et al., J. Immunol. 136:2348, 1986), have been implicated in mediating many of autoimmune diseases, including multiple sclerosis (MS), rheumatoid arthritis (RA), type 1, or insulin dependent, diabetes (IDDM),

5 inflammatory bowel disease (IBD), and psoriasis (Mosmann and Sad, Immunol. Today 17:138, 1996). Thus, antagonism of a Th1-promoting cytokine such as IL-18 would be expected to inhibit disease development. IL-18 specific mAbs could be used as an antagonist.

The role of IL-18 in the development of autoimmune diseases has been  
10 demonstrated. Accordingly, it has been demonstrated that IL-18 expression is significantly increased in the pancreas and spleen of the nonobese diabetic (NOD) mouse immediately prior to the onset of disease (Rothe et al., J. Clin. Invest. 99:469, 1997). Similarly, IL-18 levels have been shown to be markedly elevated in the synovial fluid of rheumatoid arthritis patients (Kawashima et al., Arthritis and  
15 Rheumatism 39:598, 1996). Furthermore, it has been demonstrated that IL-18 administration increases the clinical severity of murine experimental allergic encephalomyelitis (EAE), a Th1-mediated autoimmune disease that is a model for multiple sclerosis. In addition, it has been shown that neutralizing anti-rat IL-18 antiserum prevents the development of EAE in female Lewis rats (Wildbaum et al., J.  
20 Immunol. 161:6368, 1998). Accordingly, IL-18 is a desirable target for the development of a novel therapeutic for autoimmunity.

Taniguchi et al., J. Immunol. Methods 206:107, describe seven murine and six rat anti-human IL-18 mAbs, which bind to four distinct antigenic sites. One of the murine mAbs (#125-2H), and the six rat mAbs inhibit IL-18-induced IFN- $\gamma$   
25 production by KG-1 cells, with the rat mAbs exhibiting neutralizing activities 10-fold lower than that of #125-2H. As demonstrated by Western blot analysis, three of the murine mAbs, but none of the rat mAbs, are strongly reactive with membrane-bound human IL-18. In addition, an enzyme-linked immunosorbent assay (ELISA) to detect human IL-18 is described, utilizing #125-2H and a rat mAb. The limit of  
30 detection of this ELISA is 10 pg/ml.

European patent application EP 0 712 931 discloses two mouse anti-human IL-18 mAbs, H1 (IgG1) and H2 (IgM). As demonstrated by Western blot analysis, both mAbs react with membrane-bound human IL-18, but not with membrane-bound human IL-12. H1 is utilized in an immunoaffinity chromatography protocol  
35 to purify human IL-18, and in an ELISA to measure human IL-18. H2 is utilized in a radioimmunoassay to measure human IL-18.

Neutralizing IL-18 antibodies may potentially be useful in relieving autoimmune diseases and related symptoms in man. Hence there is a need in the art for a high affinity IL-18 antagonist, such as a neutralizing monoclonal antibody to human interleukin 18, which would reduce Th1 differentiation and proliferation and thus autoimmune diseases and related symptoms.

#### SUMMARY OF THE INVENTION

In a first aspect, the present invention provides rodent (e.g., rat and murine) neutralizing monoclonal antibodies specific for human interleukin-18 and having a binding affinity characterized by a dissociation constant equal to or less than about  $3.9 \times 10^{-11}$  M as described in the detailed description. Exemplary of such monoclonal antibodies are the rat monoclonal antibody 2C10 and rat and murine monoclonal antibodies such as 14B7 and 13G9. Another aspect of the invention are hybridomas such as 19522C10(2)F2(1)A1, 195214B7(1)H10 and 187413G9(3)F12

In a related aspect, the present invention provides neutralizing Fab fragments or  $F(ab')_2$  fragments thereof specific for human interleukin-18 produced by deleting the Fc region of the rodent neutralizing monoclonal antibodies of the present invention.

In still another related aspect, the present invention provides an altered antibody specific for human interleukin-18 which comprises complementarity determining regions (CDRs) derived from a non-human neutralizing monoclonal antibody (mAb) characterized by a dissociation constant equal to or less than about  $3.9 \times 10^{-11}$  M for human interleukin-18 and nucleic acid molecules encoding the same. When the altered antibody is a humanized antibody, the sequences that encode complementarity determining regions (CDRs) from a non-human immunoglobulin are inserted into a first immunoglobulin partner in which at least one, and preferably all complementarity determining regions (CDRs) of the first immunoglobulin partner are replaced by CDRs from the non-human monoclonal antibody. Preferably, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner as well, which comprises all or a part of an immunoglobulin constant chain.

In a related aspect, the present invention provides CDRs derived from non-human neutralizing monoclonal antibodies (mAbs) characterized by a dissociation constant equal to or less than about  $3.9 \times 10^{-11}$  M for human interleukin-18, and nucleic acid molecules encoding such CDRs.

In still another aspect, there is provided a chimeric antibody containing human heavy and light chain constant regions and heavy and light chain variable

regions derived from non-human neutralizing monoclonal antibodies characterized by a dissociation constant equal to or less than about  $3.9 \times 10^{-11}$  M for human interleukin-18.

In yet another aspect, the present invention provides a pharmaceutical composition which contains one (or more) of the above described altered antibodies and a pharmaceutically acceptable carrier.

In a further aspect, the present invention provides a method for treating conditions in humans associated with excess Th1 production, for example autoimmune diseases, by administering to said human an effective amount of the pharmaceutical composition of the invention.

In yet another aspect, the present invention provides methods for, and components useful in, the recombinant production of altered antibodies (e.g., engineered antibodies, CDRs, Fab or F(ab)<sub>2</sub> fragments, or analogs thereof) which are derived from non-human neutralizing monoclonal antibodies (mAbs) characterized by a dissociation constant equal to or less than  $3.9 \times 10^{-11}$  M for human IL-18. These components include isolated nucleic acid sequences encoding same, recombinant plasmids containing the nucleic acid sequences under the control of selected regulatory sequences which are capable of directing the expression thereof in host cells (preferably mammalian) transfected with the recombinant plasmids. The production method involves culturing a transfected host cell line of the present invention under conditions such that an altered antibody, preferably a humanized antibody, is expressed in said cells and isolating the expressed product therefrom.

In yet another aspect of the invention is a method to diagnose conditions associated with excess Th1 production in a human which comprises obtaining a sample of biological fluid from a patient and allowing the antibodies and altered antibodies of the instant invention to come in contact with such sample under conditions such that an IL-18/antibody (monoclonal or altered) complex is formed and detecting the presence or absence of said IL-18/antibody complex.

Other aspects and advantages of the present invention are described further in the detailed description and the preferred embodiments thereof.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 [SEQ ID NOS: 1 and 2] illustrates the light chain variable region for the rat antibody 2C10. Fig. 1 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 3-8]. The bolded area indicates the degenerate primer sequence.

Fig. 2. [SEQ ID NOS: 9 and 10] illustrates the heavy chain variable region for the rat antibody 2C10. Fig. 2 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 11-16]. The bolded area indicates the degenerate primer sequence.

5 Fig. 3 [SEQ ID NOS: 17 and 18] illustrates the light chain variable region for the murine antibody 13G9. Fig. 3 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 19-24]. The bolded area indicates the degenerate primer sequence.

10 Fig 4 [SEQ ID NOS: 25 and 26] illustrates the heavy chain variable region for the murine antibody 13G9. Fig. 4 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 27-32]. The bolded area indicates the degenerate primer sequence.

15 Fig 5 [SEQ ID NOS: 33 and 34] illustrates the light chain variable region for the rat antibody 14B7. Fig. 5 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 35-40]. The bolded area indicates the degenerate primer sequence.

20 Fig 6 [SEQ ID NOS: 41 and 42] illustrates the heavy chain variable region for the rat antibody 14B7. Fig. 6 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 43-48]. The bolded area indicates the degenerate primer sequence.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a variety of antibodies, altered antibodies and fragments thereof, which are characterized by human IL-18 binding specificity, neutralizing activity, and high affinity for human IL-18 as exemplified in rat  
25 monoclonal antibody 2C10, murine monoclonal antibody 13G9 and rat monoclonal antibody 14B7. The antibodies of the present invention were prepared by conventional hybridoma techniques to generate novel neutralizing antibodies. These products are useful in therapeutic and pharmaceutical compositions for treating IL-  
30 18-mediated disorders, e.g. autoimmune diseases, including multiple sclerosis (MS), rheumatoid arthritis (RA), type 1, or insulin dependent, diabetes (IDDM), inflammatory bowel disease (IBD), and psoriasis (Mosmann and Sad, Immunol. Today 17:138, 1996). These products are also useful in the diagnosis of IL-18-mediated conditions by measurement (e.g., enzyme linked immunosorbent assay  
35 (ELISA)) of endogenous IL-18 levels in humans or IL-18 released *ex vivo* from activated cells.

*I. Definitions.*

"Altered antibody" refers to a protein encoded by an altered immunoglobulin coding region, which may be obtained by expression in a selected host cell. Such altered antibodies are engineered antibodies (e.g., chimeric or humanized antibodies) or antibody fragments lacking all or part of an immunoglobulin constant region, e.g., Fv, Fab, or F(ab)<sub>2</sub> and the like.

"Altered immunoglobulin coding region" refers to a nucleic acid sequence encoding altered antibody of the invention. When the altered antibody is a CDR-grafted or humanized antibody, the sequences that encode the complementarity determining regions (CDRs) from a non-human immunoglobulin are inserted into a first immunoglobulin partner comprising human variable framework sequences. Optionally, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner.

"First immunoglobulin partner" refers to a nucleic acid sequence encoding a human framework or human immunoglobulin variable region in which the native (or naturally-occurring) CDR-encoding regions are replaced by the CDR-encoding regions of a donor antibody. The human variable region can be an immunoglobulin heavy chain, a light chain (or both chains), an analog or functional fragments thereof. Such CDR regions, located within the variable region of antibodies (immunoglobulins) can be determined by known methods in the art. For example Kabat et al. (Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)) disclose rules for locating CDRs. In addition, computer programs are known which are useful for identifying CDR regions/structures.

"Neutralizing" refers to an antibody that inhibits IL-18 activity by preventing the binding of human IL-18 to its specific receptor or by inhibiting the signaling of IL-18 through its receptor, should binding occur. A mAb is neutralizing if it is 90% effective, preferably 95% effective and most preferably 100% effective in inhibiting IL-18 activity as measured in the IL-18 neutralization assay, see Example 1 and Table I.

The term "high affinity" refers to an antibody having a binding affinity characterized by a K<sub>d</sub> equal to or less than  $3.9 \times 10^{-11}$  M for human IL-18 as determined by optical biosensor analysis (see Example 2 and Table I).

By "binding specificity for human IL-18" is meant a higher affinity for human IL-18 than murine, or other IL-18.

"Second immunoglobulin partner" refers to another nucleotide sequence encoding a protein or peptide to which the first immunoglobulin partner is fused in



frame or by means of an optional conventional linker sequence (i.e., operatively linked). Preferably it is an immunoglobulin gene. The second immunoglobulin partner may include a nucleic acid sequence encoding the entire constant region for the same (i.e., homologous - the first and second altered antibodies are derived from the same source) or an additional (i.e., heterologous) antibody of interest. It may be an immunoglobulin heavy chain or light chain (or both chains as part of a single polypeptide). The second immunoglobulin partner is not limited to a particular immunoglobulin class or isotype. In addition, the second immunoglobulin partner may comprise part of an immunoglobulin constant region, such as found in a Fab, or F(ab)<sub>2</sub> (i.e., a discrete part of an appropriate human constant region or framework region). Such second immunoglobulin partner may also comprise a sequence encoding an integral membrane protein exposed on the outer surface of a host cell, e.g., as part of a phage display library, or a sequence encoding a protein for analytical or diagnostic detection, e.g., horseradish peroxidase, β-galactosidase, etc.

The terms Fv, Fc, Fd, Fab, or F(ab)<sub>2</sub> are used with their standard meanings (see, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, (1988)).

As used herein, an "engineered antibody" describes a type of altered antibody, i.e., a full-length synthetic antibody (e.g., a chimeric or humanized antibody as opposed to an antibody fragment) in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous parts from one or more donor antibodies which have specificity for the selected epitope. For example, such molecules may include antibodies characterized by a humanized heavy chain associated with an unmodified light chain (or chimeric light chain), or vice versa. Engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the acceptor antibody light and/or heavy variable domain framework regions in order to retain donor antibody binding specificity. These antibodies can comprise replacement of one or more CDRs (preferably all) from the acceptor antibody with CDRs from a donor antibody described herein.

A "chimeric antibody" refers to a type of engineered antibody which contains naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more)

human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al., Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)).

The term "donor antibody" refers to an antibody (monoclonal, or  
5 recombinant) which contributes the nucleic acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody. One donor antibody suitable for use in this  
10 invention is a non-human neutralizing monoclonal antibody (i.e., rat) designated as 2C10. The antibody 2C10 is defined as a high affinity, human-IL-18 specific (i.e., does not recognize murine IL-18), neutralizing antibody of isotype IgG<sub>1,k</sub> having the variable light chain DNA and amino acid sequences of SEQ ID NOs: 1 and 2 and respectively, the variable heavy chain DNA and amino acid sequences of SEQ ID  
15 NOs: 9 and 10 on a suitable murine IgG constant region.

The term "acceptor antibody" refers to an antibody (monoclonal, or recombinant) heterologous to the donor antibody, which contributes all (or any portion, but preferably all) of the nucleic acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to  
20 the first immunoglobulin partner. Preferably a human antibody is the acceptor antibody.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of  
25 Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate).

30 CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include analogs of the naturally occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they  
35 were derived.

By sharing the antigen binding specificity or neutralizing ability is meant, for example, that although mAb 2C10 may be characterized by a certain level of antigen

affinity, a CDR encoded by a nucleic acid sequence of 2C10 in an appropriate structural environment may have a lower, or higher affinity. It is expected that CDRs of 2C10 in such environments will nevertheless recognize the same epitope(s) as 2C10. Exemplary light chain CDRs of 2C10 include

5 SEQ ID NO: 3;

SEQ ID NO: 5;

SEQ ID NO: 7;

and exemplary heavy chain CDRs of 2C10 include

SEQ ID NO: 11;

10 SEQ ID NO: 13;

and SEQ ID NO: 15.

A "functional fragment" is a partial heavy or light chain variable sequence (e.g., minor deletions at the amino or carboxy terminus of the immunoglobulin variable region) which retains the same antigen binding specificity and/or  
15 neutralizing ability as the antibody from which the fragment was derived.

An "analog" is an amino acid sequence modified by at least one amino acid, wherein said modification can be chemical or a substitution or a rearrangement of a few amino acids (i.e., no more than 10), which modification permits the amino acid sequence to retain the biological characteristics, e.g., antigen specificity and high  
20 affinity, of the unmodified sequence. For example, (silent) mutations can be constructed, via substitutions, when certain endonuclease restriction sites are created within or surrounding CDR-encoding regions.

Analogues may also arise as allelic variations. An "allelic variation or modification" is an alteration in the nucleic acid sequence encoding the amino acid  
25 or peptide sequences of the invention. Such variations or modifications may be due to degeneracy in the genetic code or may be deliberately engineered to provide desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

The term "effector agents" refers to non-protein carrier molecules to which  
30 the altered antibodies, and/or natural or synthetic light or heavy chains of the donor antibody or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used in the diagnostic field, e.g., polystyrene or other plastic beads, polysaccharides, e.g., as used in the BIAcore [Pharmacia] system, or other non-protein substances  
35 useful in the medical field and safe for administration to humans and animals. Other effector agents may include a macrocycle, for chelating a heavy metal atom, or

radioisotopes. Such effector agents may also be useful to increase the half-life of the altered antibodies, e.g., polyethylene glycol.

## *II. High Affinity IL-18 Monoclonal Antibodies*

5 For use in constructing the antibodies, altered antibodies and fragments of this invention, a non-human species (for example, bovine, ovine, monkey, chicken, rodent (e.g., murine and rat), etc.) may be employed to generate a desirable immunoglobulin upon presentment with native human IL-18 or a peptide epitope therefrom. Conventional hybridoma techniques are employed to provide a  
10 hybridoma cell line secreting a non-human mAb to IL-18. Such hybridomas are then screened for binding using IL-18 coated to 96-well plates, as described in the Examples section, or alternatively with biotinylated IL-18 bound to a streptavidin coated plate.

One exemplary, high affinity, neutralizing mAb of this instant invention is  
15 mAb 2C10, a rat antibody which can be used for the development of a chimeric or humanized antibody, described in more detail in examples below. The 2C10 mAb is characterized by an antigen binding specificity for human IL-18 of about  $K_d 3.9 \times 10^{-11}$  M. This mAb is characterized by being isotype IgG<sub>1,k</sub>.

Another desirable donor antibody is the murine mAb 13G9. This mAb is  
20 characterized by being isotype IgG<sub>1,k</sub>. The mAb has a dissociation constant for IL-18 of about  $12 \times 10^{-9}$  M.

Yet, another desirable donor antibody is the rat mAb, 14B7. This mAb is characterized by having a dissociation constant for IL-18 about  $1.5 \times 10^{-10}$  M. 14B7 is also characterized by being isotype IgG<sub>1,k</sub>.

25 This invention is not limited to the use of the 13G9, 2C10, 14B7, or their hypervariable (i.e., CDR) sequences. Any other appropriate high affinity IL-18 antibodies characterized by a dissociation constant equal or less than about  $3.9 \times 10^{-11}$  M for human IL-18 and corresponding anti-IL-18 CDRs may be substituted therefor. Wherever in the following description the donor antibody is identified as 13G9,  
30 2C10, 14B7, this designation is made for illustration and simplicity of description only.

### III. Antibody Fragments

The present invention also includes the use of Fab fragments or F(ab')<sub>2</sub> fragments derived from mAbs directed against human IL-18. These fragments are useful as agents protective *in vivo* against IL-18 and Th1-mediated conditions or *in vitro* as part of an IL-18 diagnostic. A Fab fragment contains the entire light chain and amino terminal portion of the heavy chain; and an F(ab')<sub>2</sub> fragment is the fragment formed by two Fab fragments bound by disulfide bonds. MAbs 13G9, 2C10, 14B7, and other similar high affinity, IL-18 binding antibodies, provide sources of Fab fragments and F(ab')<sub>2</sub> fragments which can be obtained by conventional means, e.g., cleavage of the mAb with the appropriate proteolytic enzymes, papain and/or pepsin, or by recombinant methods. These Fab and F(ab')<sub>2</sub> fragments are useful themselves as therapeutic, prophylactic or diagnostic agents, and as donors of sequences including the variable regions and CDR sequences useful in the formation of recombinant or humanized antibodies as described herein.

The Fab and F(ab')<sub>2</sub> fragments can be constructed via a combinatorial phage library (see, e.g., Winter et al., Ann. Rev. Immunol., 12:433-455 (1994)) or via immunoglobulin chain shuffling (see, e.g., Marks et al., Bio/Technology, 10:779-783 (1992), which are both hereby incorporated by reference in their entirety) wherein the Fd or v<sub>H</sub> immunoglobulin from a selected antibody (e.g., 13G9) is allowed to associate with a repertoire of light chain immunoglobulins, v<sub>L</sub> (or v<sub>K</sub>), to form novel Fabs. Conversely, the light chain immunoglobulin from a selected antibody may be allowed to associate with a repertoire of heavy chain immunoglobulins, v<sub>H</sub> (or Fd), to form novel Fabs.

### IV. Anti-IL-18 Amino Acid and Nucleotide Sequences of Interest

The mAb 2C10 or other antibodies described above may contribute sequences, such as variable heavy and/or light chain peptide sequences, framework sequences, CDR sequences, functional fragments, and analogs thereof, and the nucleic acid sequences encoding them, useful in designing and obtaining various altered antibodies which are characterized by the antigen binding specificity of the donor antibody.

As one example, the present invention provides variable light chain and variable heavy chain sequences from the IL-18 mAb 2C10 and sequences derived therefrom.

The nucleic acid sequences of this invention, or fragments thereof, encoding the variable light chain and heavy chain peptide sequences are also useful for mutagenic introduction of specific changes within the nucleic acid sequences

encoding the CDRs or framework regions, and for incorporation of the resulting modified or fusion nucleic acid sequence into a plasmid for expression..

Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which encode the variable heavy and light chain amino acid sequences, and CDR sequences of the invention as well as functional fragments and analogs thereof which share the antigen specificity of the donor antibody. The isolated nucleic acid sequences of this invention, or fragments thereof, encoding the variable chain peptide sequences or CDRs can be used to produce altered antibodies, e.g., chimeric or humanized antibodies, or other engineered antibodies of this invention when operatively combined with a second immunoglobulin partner.

It should be noted that in addition to isolated nucleic acid sequences encoding portions of the altered antibody and antibodies described herein, other such nucleic acid sequences are encompassed by the present invention, such as those complementary to the native CDR-encoding sequences or complementary to the modified human framework regions surrounding the CDR-encoding regions. Useful DNA sequences include those sequences which hybridize under stringent hybridization conditions [see, T. Maniatis et al, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA sequences. An example of one such stringent hybridization condition is hybridization at 4XSSC at 65°C, followed by a washing in 0.1XSSC at 65°C for an hour. Alternatively an exemplary stringent hybridization condition is in 50% formamide, 4XSSC at 42°C. Preferably, these hybridizing DNA sequences are at least about 18 nucleotides in length, i.e., about the size of a CDR.

25

#### V. *Altered Immunoglobulin Molecules And Altered Antibodies*

Altered immunoglobulin molecules can encode altered antibodies which include engineered antibodies such as chimeric antibodies and humanized antibodies. A desired altered immunoglobulin coding region contains CDR-encoding regions that encode peptides having the antigen specificity of an IL-18 antibody, preferably a high affinity antibody such as provided by the present invention, inserted into a first immunoglobulin partner (a human framework or human immunoglobulin variable region).

Preferably, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner. The second immunoglobulin partner is defined above, and may include a sequence encoding a second antibody region of interest, for example an Fc region. Second immunoglobulin partners may also include sequences

encoding another immunoglobulin to which the light or heavy chain constant region is fused in frame or by means of a linker sequence. Engineered antibodies directed against functional fragments or analogs of IL-18 may be designed to elicit enhanced binding with the same antibody.

5       The second immunoglobulin partner may also be associated with effector agents as defined above, including non-protein carrier molecules, to which the second immunoglobulin partner may be operatively linked by conventional means.

      Fusion or linkage between the second immunoglobulin partners, e.g., antibody sequences, and the effector agent may be by any suitable means, e.g., by  
10       conventional covalent or ionic bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, and the like. Such techniques are known in the art and readily described in conventional chemistry and biochemistry texts.

      Additionally, conventional linker sequences which simply provide for a desired amount of space between the second immunoglobulin partner and the  
15       effector agent may also be constructed into the altered immunoglobulin coding region. The design of such linkers is well known to those of skill in the art.

      In addition, signal sequences for the molecules of the invention may be modified to enhance expression.

      An exemplary altered antibody contains a variable heavy and/or light chain  
20       peptide or protein sequence having the antigen specificity of mAb 2C10, e.g., the V<sub>H</sub> and V<sub>L</sub> chains. Still another desirable altered antibody of this invention is characterized by the amino acid sequence containing at least one, and preferably all of the CDRs of the variable region of the heavy and/or light chains of the rat antibody molecule 2C10 with the remaining sequences being derived from a human  
25       source, or a functional fragment or analog thereof.

      In still a further embodiment, the engineered antibody of the invention may have attached to it an additional agent. For example, the procedure of recombinant DNA technology may be used to produce an engineered antibody of the invention in which the Fc fragment or CH2 CH3 domain of a complete antibody molecule has  
30       been replaced by an enzyme or other detectable molecule (i.e., a polypeptide effector or reporter molecule).

      The second immunoglobulin partner may also be operatively linked to a non-immunoglobulin peptide, protein or fragment thereof heterologous to the CDR-containing sequence, for example, having the antigen specificity of rat 2C10. The  
35       resulting protein may exhibit both anti-IL-18 antigen specificity and characteristics of the non-immunoglobulin upon expression. That fusion partner characteristic may be, e.g., a functional characteristic such as another binding or receptor domain, or a

therapeutic characteristic if the fusion partner is itself a therapeutic protein, or additional antigenic characteristics.

Another desirable protein of this invention may comprise a complete antibody molecule, having full length heavy and light chains, or any discrete  
5 fragment thereof, such as the Fab or F(ab')<sub>2</sub> fragments, a heavy chain dimer, or any minimal recombinant fragments thereof such as an F<sub>v</sub> or a single-chain antibody (SCA) or any other molecule with the same specificity as the selected donor mAb, e.g., mAb 2C10. Such protein may be used in the form of an altered antibody, or may be used in its unfused form.

10 Whenever the second immunoglobulin partner is derived from an antibody different from the donor antibody, e.g., any isotype or class of immunoglobulin framework or constant regions, an engineered antibody results. Engineered antibodies can comprise immunoglobulin (Ig) constant regions and variable  
15 framework regions from one source, e.g., the acceptor antibody, and one or more (preferably all) CDRs from the donor antibody, e.g., the anti-IL-18 antibody described herein. In addition, alterations, e.g., deletions, substitutions, or additions, of the acceptor mAb light and/or heavy variable domain framework region at the nucleic acid or amino acid levels, or the donor CDR regions may be made in order to retain donor antibody antigen binding specificity.

20 Such engineered antibodies are designed to employ one (or both) of the variable heavy and/or light chains of the IL-18 mAb (optionally modified as described) or one or more of the below-identified heavy or light chain CDRs. The engineered antibodies would be expected to be neutralizing, i.e., they desirably block binding to the receptor of the IL-18 protein and they also block or prevent  
25 proliferation of IL-18 dependent cells.

Such engineered antibodies may include a humanized antibody containing the framework regions of a selected human immunoglobulin or subtype, or a chimeric antibody containing the human heavy and light chain constant regions fused to the IL-18 antibody functional fragments. A suitable human (or other  
30 animal) acceptor antibody may be one selected from a conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant  
35 region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted



that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody.

Desirably the heterologous framework and constant regions are selected from human immunoglobulin classes and isotypes, such as IgG (subtypes 1 through 4), IgM, IgA, and IgE. However, the acceptor antibody need not comprise only human immunoglobulin protein sequences. For instance a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding a non-immunoglobulin amino acid sequence such as a polypeptide effector or reporter molecule.

One example of a particularly desirable humanized antibody would contain CDRs of 2C10 inserted onto the framework regions of a selected human antibody sequence. For neutralizing humanized antibodies, one, two or preferably three CDRs from the IL-18 antibody heavy chain and/or light chain variable regions are inserted into the framework regions of the selected human antibody sequence, replacing the native CDRs of the latter antibody.

Preferably, in a humanized antibody, the variable domains in both human heavy and light chains have been engineered by one or more CDR replacements. It is possible to use all six CDRs, or various combinations of less than the six CDRs. Preferably all six CDRs are replaced. It is possible to replace the CDRs only in the human heavy chain, using as light chain the unmodified light chain from the human acceptor antibody. Still alternatively, a compatible light chain may be selected from another human antibody by recourse to the conventional antibody databases. The remainder of the engineered antibody may be derived from any suitable acceptor human immunoglobulin.

The engineered humanized antibody thus preferably has the structure of a natural human antibody or a fragment thereof, and possesses the combination of properties required for effective therapeutic use, e.g., treatment of IL-18 mediated inflammatory diseases in man, or for diagnostic uses.

As another example, an engineered antibody may contain CDRs of the variable light chain region of 2C10 and CDRs of the variable heavy chain region of 13G9. The resulting humanized antibody should be characterized by the same antigen binding specificity and high affinity of mAb 2C10.

It will be understood by those skilled in the art that an engineered antibody may be further modified by changes in variable domain amino acids without necessarily affecting the specificity and high affinity of the donor antibody (i.e., an analog). It is anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the variable domain frameworks or CDRs or both.

In addition, the constant region may be altered to enhance or decrease selective properties of the molecules of the instant invention. For example, dimerization, binding to Fc receptors, or the ability to bind and activate complement (see, e.g., Angal et al., Mol. Immunol. 30:105-108 (1993), Xu et al., J. Biol. Chem., 269:3469-3474 (1994), Winter et al., EP 307,434-B).

An altered antibody which is a chimeric antibody differs from the humanized antibodies described above by providing the entire non-human donor antibody heavy chain and light chain variable regions, including framework regions, in association with human immunoglobulin constant regions for both chains. It is anticipated that chimeric antibodies which retain additional non-human sequence relative to humanized antibodies of this invention may elicit a significant immune response in humans.

Such antibodies could be useful in the prevention and treatment of IL-18 mediated disorders, as discussed below.

15

#### *VI. Production Of Altered Antibodies And Engineered Antibodies*

Preferably, the variable light and/or heavy chain sequences and the CDRs of mAb 2C10 or other suitable donor mAbs, and their encoding nucleic acid sequences, are utilized in the construction of altered antibodies, preferably humanized antibodies, of this invention, by the following process. The same or similar techniques may also be employed to generate other embodiments of this invention.

A hybridoma producing a selected donor mAb, e.g., the rat antibody 2C10, is conventionally cloned, and the DNA of its heavy and light chain variable regions obtained by techniques known to one of skill in the art, e.g., the techniques described in Sambrook *et al.*, (Molecular Cloning (A Laboratory Manual), 2nd edition, Cold Spring Harbor Laboratory (1989)). The variable heavy and light regions of 2C10 containing at least the CDR-encoding regions and those portions of the acceptor mAb light and/or heavy variable domain framework regions required in order to retain donor mAb binding specificity, as well as the remaining immunoglobulin-derived parts of the antibody chain derived from a human immunoglobulin can be obtained using polynucleotide primers and reverse transcriptase. The CDR-encoding regions are identified using a known database and by comparison to other antibodies.

A rat/human chimeric antibody may then be prepared and assayed for binding ability. Such a chimeric antibody contains the entire non-human donor antibody  $V_H$  and  $V_L$  regions, in association with human Ig constant regions for both chains.

A humanized antibody may be derived from the chimeric antibody, or preferably, made synthetically by inserting the donor mAb CDR-encoding regions from the heavy and light chains appropriately within the selected heavy and light chain framework. Alternatively, a humanized antibody of the invention may be prepared using standard mutagenesis techniques. Thus, the resulting humanized antibody contains human framework regions and donor mAb CDR-encoding regions. There may be subsequent manipulation of framework residues. The resulting humanized antibody can be expressed in recombinant host cells, e.g., COS, CHO or myeloma cells. Other humanized antibodies may be prepared using this technique on other suitable IL-18-specific, neutralizing, high affinity, non-human antibodies.

A conventional expression vector or recombinant plasmid can be produced by placing these coding sequences for the altered antibody in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g., CMV promoter, and signal sequences, which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antibody light or heavy chain. Preferably this second expression vector is identical to the first except insofar as the coding sequences and selectable markers are concerned, so to ensure as far as possible that each polypeptide chain is functionally expressed. Alternatively, the heavy and light chain coding sequences for the altered antibody may reside on a single vector.

A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention. The humanized antibody which includes the association of both the recombinant heavy chain and/or light chain is screened from culture by appropriate assay, such as ELISA or RIA. Similar conventional techniques may be employed to construct other altered antibodies and molecules of this invention.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the conventional pUC series of cloning vectors, may be used. One vector used is pUC19, which is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or

Pharmacia (Uppsala, Sweden). Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

5           Similarly, the vectors employed for expression of the engineered antibodies according to this invention may be selected by one of skill in the art from any conventional vector. The vectors also contain selected regulatory sequences (such as CMV promoters) which direct the replication and expression of heterologous DNA sequences in selected host cells. These vectors contain the above described DNA  
10 sequences which code for the engineered antibody or altered immunoglobulin coding region. In addition, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

          The expression vectors may also be characterized by genes suitable for  
15 amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

20           The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in  
25 the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

          The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the engineered antibodies or altered immunoglobulin molecules thereof. Host cells useful for the cloning and  
30 other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of *E. coli* are used for replication of the cloning vectors and other steps in the construction of altered antibodies of this invention.

          Suitable host cells or cell lines for the expression of the engineered antibody or altered antibody of the invention are preferably mammalian cells such as CHO,  
35 COS, a fibroblast cell (e.g., 3T3), and myeloid cells, and more preferably a CHO or a myeloid cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be

employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook *et al.*, cited above.

5 Bacterial cells may prove useful as host cells suitable for the expression of the recombinant Fabs of the present invention (see, e.g., Plückthun, A., Immunol. Rev., 130:151-188 (1992)). However, due to the tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form, any recombinant Fab produced in a bacterial cell would have to be screened for retention of antigen binding ability. If the molecule expressed by the  
10 bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host. For example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be employed in this method.

Where desired, strains of yeast cells known to those skilled in the art are also  
15 available as host cells, as well as insect cells, e.g. *Drosophila* and *Lepidoptera* and viral expression systems. See, e.g. Miller *et al.*, Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein.

The general methods by which the vectors of the invention may be constructed, the transfection methods required to produce the host cells of the  
20 invention, and culture methods necessary to produce the altered antibody of the invention from such host cell are all conventional techniques. Likewise, once produced, the altered antibodies of the invention may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis  
25 and the like. Such techniques are within the skill of the art and do not limit this invention.

Yet another method of expression of the humanized antibodies may utilize expression in a transgenic animal, such as described in U.S. Patent No. 4,873,316. This relates to an expression system using the animal's casein promoter which when  
30 transgenically incorporated into a mammal permits the female to produce the desired recombinant protein in its milk.

Once expressed by the desired method, the engineered antibody is then examined for *in vitro* activity by use of an appropriate assay. Presently conventional ELISA assay formats are employed to assess qualitative and quantitative binding of  
35 the engineered antibody to IL-18. Additionally, other *in vitro* assays may also be used to verify neutralizing efficacy prior to subsequent human clinical studies

performed to evaluate the persistence of the engineered antibody in the body despite the usual clearance mechanisms.

Following the general procedures described for preparing humanized antibodies, one of skill in the art may also construct humanized antibodies from  
5 other donor IL-18 antibodies, variable region sequences and CDR peptides described herein. Engineered antibodies can be produced with variable region frameworks potentially recognized as "self" by recipients of the engineered antibody. Minor modifications to the variable region frameworks can be implemented to effect large increases in antigen binding without appreciable increased immunogenicity for the  
10 recipient. Such engineered antibodies may effectively treat a human for IL-18 mediated conditions. Such antibodies may also be useful in the diagnosis of such conditions.

#### VII. *Therapeutic/Prophylactic Uses*

15 This invention also relates to a method of treating humans experiencing autoimmune related symptoms, such as MS, which comprises administering an effective dose of antibodies including one or more of the engineered antibodies or altered antibodies described herein, or fragments thereof.

The therapeutic response induced by the use of the molecules of this  
20 invention is produced by the binding to human IL-18 and thus subsequently blocking Th1 stimulation. Thus, the molecules of the present invention, when in preparations and formulations appropriate for therapeutic use, are highly desirable for those persons experiencing autoimmune disease, such as but not limited to MS, RA, IDDM, IBD and psoriasis.

25 The altered antibodies, antibodies and fragments thereof of this invention may also be used in conjunction with other antibodies, particularly human mAbs reactive with other markers (epitopes) responsible for the condition against which the engineered antibody of the invention is directed.

The therapeutic agents of this invention are believed to be desirable for  
30 treatment of autoimmune conditions from about 2 days to 6 months or as needed. For example, longer treatments may be desirable when treating MS or the like. The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient.

35 The mode of administration of the therapeutic agent of the invention may be any suitable route which delivers the agent to the host. The altered antibodies, antibodies, engineered antibodies, and fragments thereof, and pharmaceutical

compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly, intravenously, or intranasally.

Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the engineered (e.g., humanized) antibody of the invention as an active ingredient in a pharmaceutically acceptable carrier. In the prophylactic agent of the invention, an aqueous suspension or solution containing the engineered antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the engineered antibody of the invention or a cocktail thereof dissolved in an pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.4% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antibody of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of an engineered antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 30 and preferably 5 mg to about 25 mg of an engineered antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania.

It is preferred that the therapeutic agent of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. To effectively treat an inflammatory disorder in a human or other animal, one dose of approximately 0.1 mg to approximately 20 mg per 70 kg body weight of a protein or an antibody of this invention should be administered parenterally, preferably *i.v.*

or i.m. (intramuscularly). Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician during the disease.

The altered antibodies and engineered antibodies of this invention may also be used in diagnostic regimens, such as for the determination of IL-18 mediated disorders or tracking progress of treatment of such disorders. As diagnostic reagents, these altered antibodies may be conventionally labeled for use in ELISA's and other conventional assay formats for the measurement of IL-18 levels in serum, plasma or other appropriate tissue, or the release by human cells in culture. The nature of the assay in which the altered antibodies are used are conventional and do not limit this disclosure.

Thus, one embodiment of the present invention relates to a method for aiding the diagnosis of autoimmune disease and other conditions associated with excess Th1 T cell production in a patient which comprises the steps of determining the amount of human IL-18 in sample (plasma or tissue) obtained from said patient and comparing said determined amount to the mean amount of human IL-18 in the normal population, whereby the presence of a significantly elevated amount of IL-18 in the patient's sample is an indication autoimmune disease and other conditions associated with excess Th1 T cell production.

The antibodies, altered antibodies or fragments thereof described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

The following examples illustrate various aspects of this invention including the construction of exemplary engineered antibodies and expression thereof in suitable vectors and host cells, and are not to be construed as limiting the scope of this invention. All amino acids are identified by conventional three letter or single letter codes. All necessary restriction enzymes, plasmids, and other reagents and materials were obtained from commercial sources unless otherwise indicated. All general cloning legation and other recombinant DNA methodology were as performed in T. Maniatis *et al.*, cited above, or the second edition thereof (1989), eds. Sambrook *et al.*, by the same publisher ("Sambrook *et al.*").

#### Example 1 - Production of MAbs to IL-18

##### A. *Monoclonal antibody generation*

Mice (F1 hybrids of Balb/c and C57BL/6) or rats (Sprague Dawley) were immunised with 30 µg recombinant IL-18 in adjuvant and 4 weeks later with 30 µg



IL-18 in adjuvant. On the basis of a good serum antibody titre to IL-18 animals received a further immunization of 10-30 µg IL-18 (i.p. in saline). Three days following the final immunization a splenectomy was performed. Mouse or rat spleen cells were used to prepare hybridomas by standard procedures, (Zola, H.Ed.,  
5 Monoclonal Antibodies, CRC Press Inc. 1987). Positive hybridomas were cloned by the limiting dilution method.

*B. Purification of Mabs*

Mabs were purified by ProsepA (Bio Processing, Consett, UK) chromatography respectively using the manufacturer's instructions. Mabs were  
10 >95% pure by SDS-PAGE.

*C. Isotyping of Mabs*

All rat and mouse Mabs were isotyped by commercially available kits (Zymed, Amersham) and found to be IgG1 kappa.  
15

Example 2 – Assays

*A. Biotinylation Of IL-18*

IL-18 was biotinylated using a kit purchased from Molecular Probes Inc. using a 10:1 ratio of biotinylation reagent. Biotinylation had no effect on the  
20 biological activity of IL-18

*B. Hybridoma Screening Assay*

96-well plates were coated with streptavidin (2ug/ml, 100ul/well in PBS) by incubation overnight at 4°C. The solution was then aspirated and non-specific  
25 binding sites were blocked with 250µl/well of 1% bovine serum albumin (BSA) in TBS buffer (50mM Tris, 150 mM NaCl, 0.02% Kathon, pH 7.4) for 5-60 minutes at RT. Following this and each of the following steps, the plate was washed 4 times in wash buffer (10 mM Tris, 150 mM NaCl, 0.05% Tween 20, 0.02% Kathon, pH 7.4). To each well, 100 µL biotin IL-18 (100ng/ml) in assay buffer (0.5% BSA, 0.05%  
30 bovine gamma globulin, 0.01% Tween 40, 20µM diethylenetriaminepentaacetic in TBS buffer) was added and the plates were incubated for 30 min at RT in a shaker-incubator. To each well 50µl hybridoma medium and 50 µl assay buffer was then

added and incubated for 60 min at RT in a shaker-incubator. To each well was then added 100ul 0.5µg/ml Eu<sup>3+</sup> labelled anti-mouse or anti-rat antibody in assay buffer. Finally 200 µl /well of enhancer (Wallac) was added and incubated for 5 min at RT and the time-resolved fluorescence measured. Hybridomas having counts >100K  
5 were expanded into 24-well plates.

### C. *Immunoassay*

To determine the specificity of the anti-IL-18 Mabs) generated 96-well plates were coated, blocked and incubated with biotin IL-18 as above. All the following  
10 incubations were performed in a shaker-incubator at RT. After washing the wells 50 µl IL-18 (3 µg/ml) or assay buffer and 50 µl Mab were added and incubated for 60 min. After washing the wells 100ul 0.5µg/ml Eu<sup>3+</sup> labelled anti-mouse or anti-rat antibody in assay buffer was added for 60 min, the wells washed and then 100 µl /well of enhancer (Wallac) was added and incubated for 5 min at RT and the time-  
15 resolved fluorescence measured. All positive hybridomas showed displacement of binding with IL-18.

### D. *Neutralization Assay*

PBMC from healthy donors were isolated by Ficol-Paque (Pharmacia)  
20 gradient and cultured in 96 well plates in 10% FBS DMEM/F12 media with 1µg/ml ConA (Sigma) in the presence of IL-18 (5ng/ml) and/or Mabs. After 18h culture at 37°C, 5% CO<sub>2</sub> in air, 90% humidity 25µl media was removed and interferon gamma (IFNg) concentration measured by immunoassay. The results, obtained from an average of three experiments, are summarized in Table I.

25

### E. *Affinity Measurements Of Monoclonal Antibody*

The affinity of the purified mAbs was measured in the BIAcore optical biosensor (Pharmacia Biosensor, Uppsala, Sweden) using a flow rate of 30ul/min. Kinetic data was evaluated using relationships described previously (Karlsson et al,  
30 J. Immunol. Meth., 145:229-240 (1991) and which is incorporated by reference in its entirety. The mAb (diluted in HBS buffer, 10 mM HEPES, 150 mM NaCl, 0.01%

Tween-20, pH 7.4) was injected over a rabbit anti-mouse IgG Fc or goat anti-rat IgG Fc surface, followed by buffer flow and the RU was recorded. IL-18 (diluted in HBS buffer) was then injected for 180 seconds followed by a buffer flow for 500 seconds and the RU was recorded. The sensor chip surface was regenerated by an injection of 0.1 M phosphoric acid. The on-rates ( $K_{\text{ass}}$ ) and off-rates ( $K_{\text{diss}}$ ) of binding were calculated using BIAcore software and together these yield a calculated equilibrium constant ( $K_D$ ) of  $12 \times 10^{-9}$  M for mAb 13G9,  $3.9 \times 10^{-11}$  M for mAb 2C10 and  $1.5 \times 10^{-10}$  M for mAb 14B7. See Table I.

#### 10 F. *Epitope Analysis Of Monoclonal Antibody*

The epitope analysis of the purified Mabs was measured in the BIAcore. Using a flow rate of 10  $\mu$ l/min, the first Mab (diluted in HBS buffer) was injected over a rabbit anti-mouse IgG Fc or goat anti-rat IgG Fc surface, followed by an injection of IL-18 for 240s, an injection of blocking Mabs for 48s and an injection of the second Mab for 240s. The surface was regenerated by an injection of 0.1M phosphoric acid and the RU was recorded after each injection. It was found that Mabs 13G9, 2C10 and 14B7 have similar or overlapping epitopes.

TABLE I

20 Affinity and neutralizing activity of mAbs reactive with human IL-18

mAb	Kd (pM) <sup>a</sup>	Neutralisation IC50 (nM) <sup>b</sup>
2C10 (rat)	39	0.1
25 14B7 (rat)	150	0.2
13G9 (mouse)	12000	3.0

<sup>a</sup> Determined by optical biosensor (BIAcore) analysis (25°C)

<sup>b</sup> Inhibition of IFN gamma production (in nM) of PBMC in response to 5ng/ml human IL-18

30

Example 3 – CDR sequences*Gene Cloning and Sequence Analysis:*

The variable heavy and light genes were cloned from hybridoma cells using standard molecular biological methods described briefly as follows. Total RNA was isolated from the hybridoma cells using TRIzol Reagent (Life Technologies Cat. # 15596-026) according to manufacturer's protocol. The RNA was reverse transcribed with a RT-PCR kit per the manufacturer's instructions (Boehringer Mannheim Cat. No. 1483-188) using a poly-dT oligonucleotide for priming. Following first strand cDNA synthesis, the heavy and light V regions were PCR amplified using 3' constant region specific primers and degenerate 5' primers. The degenerate 5' primer sequences were designed to encode the previously determined N terminal amino acid sequences of the variable heavy or light chain regions. Full length sequences from multiple clones were obtained from each PCR amplification and aligned to provide consensus. Accordingly, the first 17 bases of DNA sequence for both the heavy and light chains are PCR primer generated, however the translated protein sequence is native.

The nucleotide and deduced amino acid sequences for hybridoma antibodies 2C10, 13G9, and 14B7 are shown Figures 1-6. In each case the CDR s and the nucleotide sequences encoding them are *boxed*. The degenerate primer sequences are *bolded*.

## WHAT IS CLAIMED IS:

1. A rodent neutralizing monoclonal antibody specific for human  
5 interleukin-18 and having a binding affinity characterized by a dissociation constant  
equal to or less than about  $3.9 \times 10^{-11}$  M.
2. The monoclonal antibody according to claim 1 which is a rat  
monoclonal antibody.  
10
3. The monoclonal antibody according to claim 1 which is a murine  
monoclonal antibody.
4. The monoclonal antibody according to claim 2 which comprises the  
15 light chain amino acid sequence of SEQ ID NO: 1, and the heavy chain amino acid  
sequence of SEQ ID NO: 9.
5. The monoclonal antibody according to claim 3 which comprises the  
light chain amino acid sequence of SEQ ID NO: 17 and the heavy chain amino acid  
20 sequence of SEQ ID NO: 25.
6. The monoclonal antibody according to claim 2 which comprises the  
light chain amino acid sequence of SEQ ID NO: 33 and the heavy chain amino acid  
sequence of SEQ ID NO: 41 .  
25
7. The monoclonal antibody according to claim 1 having the identifying  
characteristics of 2C10, 14B7 or 13G9.
- 30 8. A hybridoma which produces the monoclonal antibody of claim 4.
9. A hybridoma which produces the monoclonal antibody of claim 5.
10. A hybridoma which produces the monoclonal antibody of claim 6.  
35
11. A hybridoma having the identifying characteristics of cell line  
19522C10(2)F2(1)A1, 195214B7(1)H10 and 187413G9(3)F12

12. A neutralizing Fab fragment or F(ab')<sub>2</sub> fragment thereof, produced by deleting the Fc region of the monoclonal antibody of claim 1.

5 13. An altered antibody comprising a heavy chain and a light chain, wherein the framework regions of said heavy and light chains are derived from at least one selected antibody and the amino acid sequences of the complementarity determining regions of each said chain are derived from the monoclonal antibody of claim 1.

10

14. An immunoglobulin light chain complementarity determining region (CDR), the amino acid sequence of which is selected from the group consisting of:

- (a) SEQ ID NO: 3
- (b) SEQ ID NO: 5
- 15 (c) SEQ ID NO: 7
- (d) SEQ ID NO: 19
- (e) SEQ ID NO: 21
- (f) SEQ ID NO: 23
- (g) SEQ ID NO: 35
- 20 (h) SEQ ID NO: 37
- (i) SEQ ID NO: 39

15 15. An immunoglobulin heavy chain complementarity determining region (CDR), the amino acid sequence of which is selected from the group consisting of:

- (a) SEQ ID NO: 11
- (b) SEQ ID NO: 13
- (c) SEQ ID NO: 15
- (d) SEQ ID NO: 27
- 30 (e) SEQ ID NO: 29
- (f) SEQ ID NO: 31
- (g) SEQ ID NO: 43
- (h) SEQ ID NO: 45
- (i) SEQ ID NO: 47

35

16. A nucleic acid molecule encoding the immunoglobulin complementarity determining region (CDR) of claim 14.

17. A nucleic acid molecule encoding the immunoglobulin complementarity determining region (CDR) of claim 15.

5 18. A pharmaceutical composition comprising the altered antibody of claim 13 and a pharmaceutically acceptable carrier.

19. A method of treating conditions associated with autoimmune disease comprising the step of administering to said human in need thereof an effective  
10 amount of the altered antibody of claim 13.

20. The method of claim 19 where said disease is multiple sclerosis.

15 21. The method of claim 19 where said disease is rheumatoid arthritis type I or insulin dependent diabetes.

22. The method of claim 19 where said disease is inflammatory bowel disease.

20

23. The method of claim 19 where said disease is psoriasis.

24. An isolated nucleic acid sequence which is selected from the group consisting of:

25

- (a) a nucleic acid sequence encoding the altered antibody of claim 13
- (b) a nucleic acid sequence complementary to (a); and
- (c) a fragment or analog of (a) or (b), which encodes a protein, characterized by having a specificity for human interleukin-18; wherein said sequence optionally contains a restriction site.

30

25. A method to assess the presence or absence of human IL-18 in a human which comprises obtaining a sample of biological fluid from a patient and allowing the monoclonal antibody of claim 1 to come in contact with such sample under conditions such that an IL-18/monoclonal antibody complex can form and  
35 detecting the presence or absence of said IL-18/monoclonal antibody complex.

26. A method for aiding in the diagnosis of autoimmune disease associated with comprising the steps of determining the amount of human IL-18 in a sample of a patient according to the method of claim 25 and comparing that to the mean amount of human IL-18 in the normal population, whereby the presence of
- 5 significantly elevated amount of human IL-18 in the patient is an indication of autoimmune disease.



## Vk2c10

[illegible]

# FIGURE 1

**1/6**

## VH2C10

1	E	V	Q	L	Q	Q	S	G	A	E	L	V	R	P	G	15
	GAG	GTC	CAG	CTA	CAG	CAG	TCT	GGG	GCT	GAG	CTT	GTG	AGA	CCT	GGG	
	CTC	CAG	GTC	GAT	GTC	GTC	AGA	CCC	CGA	CTC	GAA	CAC	TCT	GGA	CCC	
	100															
16	T	S	V	K	L	S	C	K	V	S	G	E	I	S	T	30
	ACC	TCT	GTG	AAG	TTA	TCT	TGC	AAA	GTT	TCT	GGC	GAA	ATA	AGT	ACA	
	TGG	AGA	CAC	TTC	AAT	AGA	ACG	TTT	CAA	AGA	CCG	CTT	TAT	TCA	TGT	
	110															
	CDRI															
31	G	Y	Y	F	H	F	V	R	R	R	P	G	Q	G	L	45
	GGA	TAC	TAT	TTC	CAC	TTT	GTG	AGG	CGA	AGG	CCT	GGA	CAG	GGT	CTG	
	CCT	ATG	ATA	AAG	GTG	AAA	CAC	TCC	GCT	TCC	GGA	CCT	GTC	CCA	GAC	
	120															
	CDRII															
46	E	W	I	G	R	I	D	P	E	D	D	S	T	K	Y	6
	GAA	TGG	ATA	GGA	AGG	ATT	GAT	CCT	GAG	GAT	GAT	AGT	ACT	AAA	TAT	
	CTT	ACC	TAT	CCT	TCC	TAA	CTA	GGA	CTC	CTA	CTA	TCA	TGA	TTT	ATA	
	130															
61	A	E	R	F	K	D	R	A	T	L	T	A	Q	T	S	75
	GCT	GAG	AGG	TTC	AAA	GAC	AGG	GCG	ACG	CTC	ACT	GCA	CAA	ACA	TCC	
	CGA	CTC	TCC	AAG	TTT	CTG	TCC	CGC	TGC	GAG	TGA	CGT	GTT	TGT	AGG	
	140															
76	S	N	T	A	Y	L	N	L	S	S	L	T	S	E	D	90
	TCC	AAC	ACA	GCC	TAC	CTG	AAC	CTC	AGC	AGC	CTG	ACC	TCT	GAG	GAC	
	AGG	TTG	TGT	CGG	ATG	GAC	TTG	GAG	TCG	TCG	GAC	TGG	AGA	CTC	CTG	
	150															
91	T	A	T	Y	F	C	T	T	W	R	I	Y	R	D	S	105
	ACT	GCA	ACT	TAT	TTT	TGT	ACC	ACA	TGG	CGG	ATA	TAC	CGA	GAT	AGT	
	TGA	CGT	TGA	ATA	AAA	ACA	TGG	TGT	ACC	GCC	TAT	ATG	GCT	CTA	TCA	
	160															
	CDRIII															
106	S	G	R	P	F	Y	V	M	D	A	W	G	Q	G	A	120
	TCT	GGC	CGC	CCC	TTC	TAT	GTT	ATG	GAT	GCC	TGG	GGT	CAA	GGA	GCT	
	AGA	CCG	GCG	GGG	AAG	ATA	CAA	TAC	CTA	CGG	ACC	CCA	GTT	CCT	CGA	
	170															
121	S	V	T	V	S	S										
	TCA	GTC	ACT	GTC	TCC	TCA										
	AGT	CAG	TGA	CAG	AGG	AGT										

FIGURE 2

Vk13g9

[illegible]

**FIGURE 3**  
**3/6**

## VH13G9

1 Q V T L K E S G P G I L K P S 15  
 CAA GTT ACT CTT AAG GAG TCT GGC CCT GGG ATA TTG AAG CCC TCA  
 GTT CAA TGA GAA TTC CTC AGA CCG GGA CCC TAT AAC TTC GGG AGT

16 Q T L S L T C S F S G F S L S 30  
 CAG ACC CTC AGT CTG ACT TGT TCT TTC TCT GGG TTT TCT CTG AGC  
 GTC TGG GAG TCA GAC TGA ACA AGA AAG AGA CCC AAA AGA GAC TCG

31 T S G M G I A W V R Q P S G K 45  
 ACT TCT GGT ATG GGT ATT GCC TGG GTT CGT CAG CCT TCA GGG AAG  
 TGA AGA CCA TAC CCA TAA CGG ACC CAA GCA GTC GGA AGT CCC TTC

46 G L E W L A D I W W D D N K Y 60  
 GGT CTG GAG TGG CTG GCA GAC ATT TGG TGG GAT GAT AAT AAG TAT  
 CCA GAC CTC ACC GAC CGT CTG TAA ACC ACC CTA CTA TTA TTC ATA

61 Y N P S L E S Q L T I S K D T 75  
 TAT AAT CCA TCC CTG GAG AGC CAG CTC ACA ATC TCC AAG GAT ACC  
 ATA TTA GGT AGG GAC CTC TCG GTC GAG TGT TAG AGG TTC CTA TGG

76 S R N Q V F L T I T S V D T A 90  
 TCC AGA AAC CAG GTA TTC CTC ACG ATC ACC AGT GTG GAC ACT GCA  
 AGG TCT TTG GTC CAT AAG GAG TGC TAG TGG TCA CAC CTG TGA CGT

91 D S A T Y Y C A R H H Y D G S 105  
 GAT TCT GCC ACT TAT TAC TGT GCT CGT CAT CAT TAC GAC GGT AGT  
 CTA AGA CGG TGA ATA ATG ACA CGA GCA GTA GTA ATG CTG CCA TCA

106 S L L P M D Y W G Q G T S V T 120  
 AGC CTC CTG CCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC  
 TCG GAG GAC GGA TAC CTG ATG ACC CCA GTT CCT TGG AGT CAG TGG

121 V S S 123  
 GTC TCC TCA  
 CAG AGG AGT

FIGURE 4

# Vk14B7

```

      1 0      30
1  D I Q M T Q S P A S L S A S L 15
   GAT ATT CAA ATG ACG CAG TCT CCA GCT TCC CTG TCT GCA TCT CTG
   CTA TAA GTT TAC TGC GTC AGA GGT CGA AGG GAC AGA CGT AGA GAC

      50      7 0      CDR I      90
16 G E T V S I E C L A S E D I Y 30
   GGA GAA ACT GTC TCC ATC GAA TGT CTA GCA AGT GAG GAC ATA TAC
   CCT CTT TGA CAG AGG TAG CTT ACA GAT CGT TCA CTC CTG TAT ATG

      1 10      13 0
31 S Y L A W Y Q Q K P G K S P Q 45
   AGT TAT TTA GCA TGG TAT CAA CAG AAG CCA GGG AAA TCT CCT CAG
   TCA ATA AAT CGT ACC ATA GTT GTC TTC GGT CCC TTT AGA GGA GTC

      150      CDR II      1 70
46 L L I Y A T K R L Q D G V P S 60
   CTC CTG ATC TAT GCC ACA AAA AGG TTG CAA GAT GGG GTC CCA TCA
   GAG GAC TAG ATA CGG TGT TTT TCC AAC GTT CTA CCC CAG GGT AGT

      19 0      210
61 R F S G S G S G T Q Y S L K I 75
   CGG TTC AGT GGC AGT GGA TCT GGC ACA CAG TAT TCT CTC AAA ATA
   GCC AAG TCA CCG TCA CCT AGA CCG TGT GTC ATA AGA GAG TTT TAT

      2 30      25 0      270
76 S D M Q P E D E G D Y F C L Q 90
   AGC GAC ATG CAA CCT GAA GAT GAA GGG GAT TAT TTC TGT CTA CAG
   TCG CTG TAC GTT GGA CTT CTA CTT CCC CTA ATA AAG ACA GAT GTC

      CDR III      2 90      31 0
91 N S K F P V T F G S G T K L E 105
   AAT TCC AAG TTT CCG GTC ACG TTC GGT TCT GGG ACC AAG CTG GAG
   TTA AGG TTC AAA GGC CAG TGC AAG CCA AGA CCC TGG TTC GAC CTC

106 I K R 108
    ATC AAA CGG
    TAG TTT GCC

```

FIGURE 5

5/6

## VH14B7

[illegible]

### FIGURE 6

## SEQUENCE LISTING

<110> Holmes, Stephen D.  
Ho, Yen Sen  
Taylor, Alexander  
Abdel-Meguid, Sherin S.

## <120> Recombinant IL-18 Antagonists Useful in Treatment of IL-18 Mediated Disorders

<130> P50897

<140> 60/125,299

<141> 1999-03-19

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<212> DNA

<213> Rattus norvegicus

<220>

<221> CDS

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Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Leu Gly  
1 5 10 15

gaa act gtc tcc atc gaa tgt ctg gca agt gag gac ata tac act tat 96  
Glu Thr Val Ser Ile Glu Cys Leu Ala Ser Glu Asp Ile Tyr Thr Tyr  
20 25 30

tta aca tgg tat cag cag aaa cca ggg aaa tct cct caa ctc ctg atc 144  
 Leu Thr Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile  
                   35                                  40                                  45

tat ggt gca aat aag ttg caa gat ggg gtc cca tca cgg ttc agt ggc 192  
 Tyr Gly Ala Asn Lys Leu Gln Asp Gly Val Pro Ser Arg Phe Ser Gly  
                   50                                  55                                  60

agt gga tct ggc aca cag tat tct ctc aag atc agc ggc ata caa cct 240  
 Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Ser Gly Ile Gln Pro  
                   65                                  70                                  75                                  80

gaa gat gaa ggg gat tat ttc tgt cta cag ggt tcc aag ttt ccg ctc 288  
 Glu Asp Glu Gly Asp Tyr Phe Cys Leu Gln Gly Ser Lys Phe Pro Leu  
   85                                  90                                  95

acg ttc ggt tct ggg acc aag ctg gag atc aaa cgg 324  
 Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg  
                                   100                                  105

<210> 2

<211> 108

<212> PRT

<213> Rattus norvegicus

<400> 2

Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Leu Gly  
   1                                  5                                  10                                  15  
 Glu Thr Val Ser Ile Glu Cys Leu Ala Ser Glu Asp Ile Tyr Thr Tyr  
                                   20                                  25                                  30  
 Leu Thr Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile  
                                   35                                  40                                  45  
 Tyr Gly Ala Asn Lys Leu Gln Asp Gly Val Pro Ser Arg Phe Ser Gly  
                                   50                                  55                                  60  
 Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Ser Gly Ile Gln Pro  
   65                                  70                                  75                                  80  
 Glu Asp Glu Gly Asp Tyr Phe Cys Leu Gln Gly Ser Lys Phe Pro Leu  
                                   85                                  90                                  95



Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg

100

105

<210> 3

<211> 33

<212> DNA

<213> Rattus norvegicus

<220>

<221> CDS

<222> (1)...(33)

<223> VK2C10 Light Chain CDR I

<400> 3

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33

Leu Ala Ser Glu Asp Ile Tyr Thr Tyr Leu Thr

1

5

10

<210> 4

<211> 11

<212> PRT

<213> Rattus norvegicus

<400> 4

Leu Ala Ser Glu Asp Ile Tyr Thr Tyr Leu Thr

1

5

10

<210> 5

<211> 21

<212> DNA

<213> Rattus norvegicus

<220>

<221> CDS

<222> (1)...(21)

<223> VK2C10 Light Chain CDR II

<400> 5

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21

Gly Ala Asn Lys Leu Gln Asp

1

5

3/21

<210> 6  
<211> 7  
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<400> 6  
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1 5

<210> 7  
<211> 27  
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<220>  
<221> CDS  
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cta cag ggt tcc aag ttt ccg ctc acg  
Leu Gln Gly Ser Lys Phe Pro Leu Thr  
1 5

27

<210> 8  
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<212> PRT  
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<400> 8  
Leu Gln Gly Ser Lys Phe Pro Leu Thr  
1 5

<210> 9  
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<220>

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<223> Heavy chain V region

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1 5 10 15	
tct gtg aag tta tct tgc aaa gtt tct ggc gaa ata agt aca gga tac	96
Ser Val Lys Leu Ser Cys Lys Val Ser Gly Glu Ile Ser Thr Gly Tyr	
20 25 30	
tat ttc cac ttt gtg agg cga agg cct gga cag ggt ctg gaa tgg ata	144
Tyr Phe His Phe Val Arg Arg Arg Pro Gly Gln Gly Leu Glu Trp Ile	
35 40 45	
gga agg att gat cct gag gat gat agt act aaa tat gct gag agg ttc	192
Gly Arg Ile Asp Pro Glu Asp Asp Ser Thr Lys Tyr Ala Glu Arg Phe	
50 55 60	
aaa gac agg gcg acg ctc act gca caa aca tcc tcc aac aca gcc tac	240
Lys Asp Arg Ala Thr Leu Thr Ala Gln Thr Ser Ser Asn Thr Ala Tyr	
65 70 75 80	
ctg aac ctc agc agc ctg acc tct gag gac act gca act tat ttt tgt	288
Leu Asn Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Thr Tyr Phe Cys	
85 90 95	
acc aca tgg cgg ata tac cga gat agt tct ggc cgc ccc ttc tat gtt	336
Thr Thr Trp Arg Ile Tyr Arg Asp Ser Ser Gly Arg Pro Phe Tyr Val	
100 105 110	
atg gat gcc tgg ggt caa gga gct tca gtc act gtc tcc tca	378
Met Asp Ala Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser	
115 120 125	

<210> 10

<211> 126

&lt;212&gt; PRT

&lt;213&gt; Rattus norvegicus

&lt;400&gt; 10

Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Thr  
 1 5 10 15  
 Ser Val Lys Leu Ser Cys Lys Val Ser Gly Glu Ile Ser Thr Gly Tyr  
 20 25 30  
 Tyr Phe His Phe Val Arg Arg Arg Pro Gly Gln Gly Leu Glu Trp Ile  
 35 40 45  
 Gly Arg Ile Asp Pro Glu Asp Asp Ser Thr Lys Tyr Ala Glu Arg Phe  
 50 55 60  
 Lys Asp Arg Ala Thr Leu Thr Ala Gln Thr Ser Ser Asn Thr Ala Tyr  
 65 70 75 80  
 Leu Asn Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Thr Tyr Phe Cys  
 85 90 95  
 Thr Thr Trp Arg Ile Tyr Arg Asp Ser Ser Gly Arg Pro Phe Tyr Val  
 100 105 110  
 Met Asp Ala Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser  
 115 120 125

&lt;210&gt; 11

&lt;211&gt; 15

&lt;212&gt; DNA

&lt;213&gt; Rattus norvegicus

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)...(15)

&lt;223&gt; VH2C10 Heavy chain CDR I

&lt;400&gt; 11

gga tac tat ttc cac  
 Gly Tyr Tyr Phe His  
 1 5

15

&lt;210&gt; 12

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Rattus norvegicus

<400> 12

Gly Tyr Tyr Phe His

1 5

<210> 13

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<213> Rattus norvegicus

<220>

<221> CDS

<222> (1)...(51)

<223> VH2C10 Heavy chain CDR II

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agg att gat cct gag gat gat agt act aaa tat gct gag agg ttc aaa 48  
Arg Ile Asp Pro Glu Asp Asp Ser Thr Lys Tyr Ala Glu Arg Phe Lys  
1 5 10 15

gac 51

<210> 14

<211> 16

<212> PRT

<213> Rattus norvegicus

<400> 14

Arg Ile Asp Pro Glu Asp Asp Ser Thr Lys Tyr Ala Glu Arg Phe Lys  
1 5 10 15

<210> 15

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<212> DNA

<213> Rattus norvegicus

<220>

<221> CDS

<222> (1)...(51)

<223> VH2C10 Heavy chain CDR III

<400> 15

7/21

tgg cgg ata tac cga gat agt tct ggc cgc ccc ttc tat gtt atg gat 48  
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 1 5 10 15

gcc 51

<210> 16

<211> 16

<212> PRT

<213> Rattus norvegicus

<400> 16

Trp Arg Ile Tyr Arg Asp Ser Ser Gly Arg Pro Phe Tyr Val Met Asp  
 1 5 10 15

<210> 17

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<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)...(342)

<223> Light chain V region

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 1 5 10 15

gat caa gcc tcc atc tct tgc aga tct agt cag agc ctt gta cac agt 96  
 Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser  
 20 25 30

aat gga aac acc tat tta cat tgg tac ctg cag aag cca ggc cag tct 144  
 Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser  
 35 40 45

cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca 192  
 Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro  
 50 55 60

gac agg ttc agt ggc agt gga tca ggt aca gat ttc aca ctc aag atc 240  
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile  
 65 70 75 80  
  
 agc aga gtg gag gct gag gat ctg gga gtt tat ttc tgc tct caa agt 288  
 Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser  
 85 90 95  
  
 aca cat gtt cct ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata 336  
 Thr His Val Pro Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile  
 100 105 110  
  
 aaa cgg 342  
 Lys Arg

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 1 5 10 15  
 Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser  
 20 25 30  
 Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser  
 35 40 45  
 Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro  
 50 55 60  
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile  
 65 70 75 80  
 Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser  
 85 90 95  
 Thr His Val Pro Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile  
 100 105 110  
 Lys Arg

<210> 19  
 <211> 48





<213> Mus musculus

<400> 22

Lys Val Ser Asn Arg Phe Ser

1

5

<210> 23

<211> 30

<212> DNA

<213> Mus musculus

<220>

<221> CDS

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<400> 23

tct caa agt aca cat gtt cct ccg tac acg

30

Ser Gln Ser Thr His Val Pro Pro Tyr Thr

1

5

10

<210> 24

<211> 10

<212> PRT

<213> Mus musculus

<400> 24

Ser Gln Ser Thr His Val Pro Pro Tyr Thr

1

5

10

<210> 25

<211> 369

<212> DNA

<213> Mus musculus

<220>

<221> CDS

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<223> Heavy chain V region

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11/21

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 1 5 10 15  
  
 acc ctc agt ctg act tgt tct ttc tct ggg ttt tct ctg agc act tct 96  
 Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser  
 20 25 30  
  
 ggt atg ggt att gcc tgg gtt cgt cag cct tca ggg aag ggt ctg gag 144  
 Gly Met Gly Ile Ala Trp Val Arg Gln Pro Ser Gly Lys Gly Leu Glu  
 35 40 45  
  
 tgg ctg gca gac att tgg tgg gat gat aat aag tat tat aat cca tcc 192  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser  
 50 55 60  
  
 ctg gag agc cag ctc aca atc tcc aag gat acc tcc aga aac cag gta 240  
 Leu Glu Ser Gln Leu Thr Ile Ser Lys Asp Thr Ser Arg Asn Gln Val  
 65 70 75 80  
  
 ttc ctc acg atc acc agt gtg gac act gca gat tct gcc act tat tac 288  
 Phe Leu Thr Ile Thr Ser Val Asp Thr Ala Asp Ser Ala Thr Tyr Tyr  
 85 90 95  
  
 tgt gct cgt cat cat tac gac ggt agt agc ctc ctg cct atg gac tac 336  
 Cys Ala Arg His His Tyr Asp Gly Ser Ser Leu Leu Pro Met Asp Tyr  
 100 105 110  
  
 tgg ggt caa gga acc tca gtc acc gtc tcc tca 369  
 Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser  
 115 120

&lt;210&gt; 26

&lt;211&gt; 123

&lt;212&gt; PRT

&lt;213&gt; Mus musculus

&lt;400&gt; 26

Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Lys Pro Ser Gln  
 1 5 10 15  
 Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser  
 12/21

	20	25	30
Gly Met Gly Ile Ala Trp Val Arg Gln Pro Ser Gly Lys Gly Leu Glu			
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Trp Leu Ala Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser			
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Leu Glu Ser Gln Leu Thr Ile Ser Lys Asp Thr Ser Arg Asn Gln Val			
65	70	75	80
Phe Leu Thr Ile Thr Ser Val Asp Thr Ala Asp Ser Ala Thr Tyr Tyr			
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Cys Ala Arg His His Tyr Asp Gly Ser Ser Leu Leu Pro Met Asp Tyr			
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<211> 48

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13/21



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 gaa act gtc tcc atc gaa tgt cta gca agt gag gac ata tac agt tat 96  
 Glu Thr Val Ser Ile Glu Cys Leu Ala Ser Glu Asp Ile Tyr Ser Tyr  
 20 25 30  
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 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile  
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 Tyr Ala Thr Lys Arg Leu Gln Asp Gly Val Pro Ser Arg Phe Ser Gly  
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 Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Ser Asp Met Gln Pro  
 65 70 75 80  
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 Glu Asp Glu Gly Asp Tyr Phe Cys Leu Gln Asn Ser Lys Phe Pro Val  
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 Tyr Ala Thr Lys Arg Leu Gln Asp Gly Val Pro Ser Arg Phe Ser Gly  
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 Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Ser Asp Met Gln Pro  
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Ser Val Lys Phe Ser Cys Lys Val Ser Gly Asp Thr Pro Thr Thr Tyr

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Tyr Val His Phe Val Arg Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile

35 40 45

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Gly Arg Ile Asp Pro Glu Asp Thr Ser Thr Lys Tyr Ala Glu Lys Phe

50 55 60

aga aat aag gcg aca ttc act gca gat cca tcc tcc aac aca gcc tac 240



Arg Asn Lys Ala Thr Phe Thr Ala Asp Pro Ser Ser Asn Thr Ala Tyr  
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acc ata atg cgg tac cat agt acc tat agg gtc tat gtt atg gat ttc 336  
Thr Ile Met Arg Tyr His Ser Thr Tyr Arg Val Tyr Val Met Asp Phe  
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Gly Arg Ile Asp Pro Glu Asp Thr Ser Thr Lys Tyr Ala Glu Lys Phe  
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Leu Asn Leu Ser Ser Leu Thr Pro Glu Asp Thr Ala Thr Tyr Phe Cys  
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<213> Rattus norvegicus

<400> 48

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/07349

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07 K 16/18; A61 K 39/395; C12 N 5/12, 15/12

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/388.1, 388.23; 435/326; 536/23.5; 424/130.1, 139.1, 141.1, 142.1, 145.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

West, US Patent full, STN via medline, caplus, embase, biosis. Search terms, interleukin-18 or IL-18, antibodies, monoclonal, humanized, 2C10, 14B7, 13G9

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	REKVIG et al. Molecular analyses of anti-DNA antibodies induced by polynavirus BK in Balb/c mice. Scandinavian Journal of Immunology. 1995, Vol.41, No.6, pages 593-602. See sequence comparison "A".	14
X	BONILLA et al. V kappa gene usage, idiotype expression and antigen binding among clones expressing the VHx24 gene family derived from naive and anti-idiotype immune Balb/c mice. Journal of Immunology. 15 July 1990, Vol.145, No.2, pages 616-622, see sequence comparison "B"	14



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

29 JUNE 2000

Date of mailing of the international search report

04 AUG 2000

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/07349

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JONES et al. Expression of TIMP3 mRNA is elevated in retinas affected by simplex retinitis pigmentosa. FEBS Letters. October 1994, Vol.352, No.2, pages 171-174, see sequence comparison "C"	15
X	EP 0528767 A1 (SANDOZ, LTD.) 24 February 1993 (24.02.93), see page 35, also see sequence comparison "D"	14
A, P	EP 0974600 A2 (KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KASAKU KENKYUJO) 26 January 2000 (26.01.2000), see entire document.	1-26
A	WO 99/09063 A1 (VEDA RESEARCH AND DEVELOPMENT CO. LTD.) 25 February 1999 (25.02.99), see entire document.	1-26

Form PCT/ISA/210 (continuation of second sheet) (July 1998) ★

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/07349

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

530/388.1, 388.23; 435/326; 536/23.5; 424/130.1, 139.1, 141.1, 142.1, 145.1

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-4, 7-8, 11-26, drawn to a rat monoclonal antibody (Rat 2C10) specific for human IL-18, a hybridoma cell line producing said antibody a nucleic acid encoding said antibody, a pharmaceutical composition comprising said antibody, a method of treatment using said antibody and a method of diagnosis using said antibody.

Group II, claims 1-3, 5, 7, 9, 11-26, drawn to a rat monoclonal antibody (Murine 13G9) specific for human IL-18, a hybridoma cell line producing said antibody a nucleic acid encoding said antibody, a pharmaceutical composition comprising said antibody, a method of treatment using said antibody and a method of diagnosis using said antibody.

Group III, claims 1-3, 6-7, 10-26, drawn to a rat monoclonal antibody (Rat 14B7) specific for human IL-18, a hybridoma cell line producing said antibody a nucleic acid encoding said antibody, a pharmaceutical composition comprising said antibody, a method of treatment using said antibody and a method of diagnosis using said antibody.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Pursuant to 37 C.F.R § 1.475 (d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first-recited product, the Rat 2C10 antibody and the nucleic acid molecule encoding it, a method of using it. Further pursuant to 37 C.F.R § 1.475 (d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/07349

methods accordingly defines a separate invention.



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